

REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-98-

0420

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1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE		3. REPORT TYPE AND DATES COVERED FINAL 01 FEB 97 TO 28 JAN 98	
4. TITLE AND SUBTITLE STRE ^{SS} -INDUCED ENHANCEMENT OF THE STARTLE REFLEX				5. FUNDING NUMBERS F49620-97-1-0006 2312/CS 61102F	
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9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) AFOSR/NL 110 Duncan Avenue Room B115 Bolling AFB DC 20332-8050 Dr Genevieve M. Haddad				10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES					
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited.				12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) A major goal of the work currently funded by the AFOSR is to determine the role of glutamate receptors in the amygdala in conditioned fear and to begin to determine brain areas involved in the inhibition of fear. We have recently found further evidence that the amygdala may be the actual storage location for fear memories and that N-methyl-D-aspartate receptors in the amygdala are critically involved in the associative process necessary for fear conditioning. We have also developed a procedure to directly measure inhibition of fear and have been using a variety of procedures to begin to determine what brain areas are activated by safety signals (stimuli which signal the absence of aversive events). We have found that safety signals uniquely induce gene expression in several brain areas which may project to and inhibit the amygdala. We have also found, contrary to prevailing dogma, that lesions of the prefrontal cortex do not affect fear inhibition. Currently we are testing the role of other brain structures (e.g., the nucleus accumbens) in fear inhibition.					
14. 19980505 047				15. NUMBER OF PAGES	
				16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT (U)		18. SECURITY CLASSIFICATION OF THIS PAGE (U)		19. SECURITY CLASSIFICATION OF ABSTRACT (U)	
20. LIMITATION OF ABSTRACT (UL)					

Final Technical Report

Grant Number: F49620-97-1-00006

Title: Stress-Induced Enhancement of the Startle Reflex

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Period of Support: This grant was originally funded for three years but was terminated on Dec. 31, 1997. Hence, this Final Technical Report contains the information sent in on Sept. 1997 plus the additional work up to Dec. 31, 1997.

A major goal of the work currently funded by the AFOSR is to determine the role of glutamate receptors in the amygdala in conditioned fear and to begin to determine brain areas involved in the inhibition of fear. We have recently found further evidence that the amygdala may be the actual storage location for fear memories and that N-methyl-D-aspartate receptors in the amygdala are critically involved in the associative process necessary for fear conditioning. We have also developed a procedure to directly measure inhibition of fear and have been using a variety of procedures to begin to determine what brain areas are activated by safety signals (stimuli which signal the absence of aversive events). We have found that safety signals uniquely induce gene expression in several brain areas which may project to and inhibit the amygdala. We have also found, contrary to prevailing dogma, that lesions of the prefrontal cortex do not affect fear inhibition. Currently we are testing the role of other brain structures (e.g., the nucleus accumbens) in fear inhibition.

1. Research Highlights

a.) Lack of a temporal gradient of retrograde amnesia following NMDA-induced lesions of the amygdala assessed with the fear-potentiated startle paradigm.

Previously we reported that electrolytic lesions of the central nucleus of the amygdala given either 6 or 30 days after training blocked the expression of potentiated startle, indicating no temporal gradient of amnesia over these intervals in this test paradigm. This contrasts with temporally graded retrograde amnesia for certain types of memory following hippocampal lesions and with amygdala lesions in avoidance learning. The present study reexamined the effects of posttraining amygdala lesions in the fear-potentiated startle test using fiber sparing, cell specific lesions of the basolateral amygdala produced by N-methyl-D-aspartate (NMDA). Like electrolytic lesions of the central nucleus, NMDA-induced lesions of the basolateral amygdala completely blocked the expression of fear-potentiated startle when performed either 6 or 30 days after training. This strengthens the possibility that fear memories may actually be stored in the amygdala.

b.) Elicitation and reduction of fear: Behavioral and neuroendocrine indices and brain induction of the immediate-early gene c-fos.

The specific neural substrates involved in Pavlovian conditioned inhibition and fear conditioning were investigated using the fear-potentiated startle paradigm and corticosterone release as indices of fear, and induction of the mRNA for the immediate-early gene c-fos as a marker of neural activity. Conditioning consisted of pairing one stimulus (CS) with footshock (US), with the footshock withheld when the CS was immediately preceded by a different modality stimulus, the conditioned inhibitor (CI). Different groups of conditioned rats were presented with either the

CS alone, the CI alone, the compound stimulus (CI/CS) or no stimuli on the test day. Naive rats, and a group of rats repeatedly exposed to the experimental cages served as controls. Rats used for c-fos in situ hybridization histochemistry were sacrificed 30 min following presentation of the first of 10 stimuli, or after cage placement. Behaviorally, the rats demonstrated reliable fear-potentiated startle to the CS, which was significantly reduced when the CS was preceded by the CI. Likewise, corticosterone release was highest in the CS group but was reduced in the group that received the compound stimulus (CI/CS). Induction of c-fos was widely observed in the brain, especially in the amygdala and hippocampus, even in unconditioned rats repeatedly exposed to the context, suggesting the involvement of these structures in a general process of attention or arousal. Conditioning specific c-fos induction (observed in the three groups that received a stimulus on the test day) was observed in the many hypothalamic areas, the medial geniculate body and the central gray (laterodorsal and ventrolateral). In addition, rats that were presented with the CS on the test day displayed the highest c-fos induction in the cingulate, infralimbic and perirhinal cortex, nucleus accumbens, lateral septum, ventral tegmental area, and dorsal cochlear nucleus. In rats that received the CI, the bed nucleus of the stria terminalis, laterodorsal and pedunculopontine tegmental nuclei, locus coeruleus and the inferior colliculus were observed to have the highest c-fos induction. These results indicate that although many brain regions are active during conditioned and unconditioned stress, there are distinct patterns observed with stimuli eliciting different behavioral/endocrine responses.

c.) Inhibition of fear-potentiated startle can be detected after the onset of a feature trained in a serial feature-negative discrimination.

Using the fear-potentiated startle paradigm in rats, 4 experiments examined whether the inhibitory effect of a feature is evident after its offset following serial feature-negative discrimination training (A+ and X - A-). When startle probes were presented shortly after the offset of X on X - A test trials, the inhibitory properties of X were observed immediately after its offset. Furthermore, trace reinforcement of X (X - +), but not delay reinforcement (X+), disrupted the ability of X to inhibit fear-potentiated startle on X - A trials. Trace conditioning to X was also retarded after A+ and X - A training. These results suggest that the inhibitory properties of the serially trained feature are present after its offset and raise the possibility that either temporal information regarding non reinforcement or poststimulus attributes of X acquire inhibitory properties.

d.) Olfactory bulbectomy enhances sensitization of the acoustic startle reflex produced by acute or repeated stress.

The effects of the olfactory bulbectomy on the acoustic startle reflex and shock-induced sensitization of the startle reflex were examined in 3 experiments. In Experiment 1, bulbectomized

animals showed a modest increase in baseline startle responding following surgery, and normal acquisition of fear-potentiated startle, but a pronounced increase in baseline startle responding during the course of conditioning relative to sham-operated controls. In Experiments 2 and 3, bulbectomized animals showed shock-induced sensitization of the startle reflex to shock intensities that did not produce sensitization in sham and unoperated controls. These data suggest that the olfactory bulbectomy results in an increase vulnerability to stressors, which may be mediated by a disinhibition of the amygdala or other structures involved in mediating stress and anxiety. Thus, the olfactory bulbectomy model of depression may share some similarities with other stress-induced models of depression.

e.) Second-order fear conditioning prevented by blocking NMDA receptors in amygdala

NMDA-type glutamate receptor antagonists disrupt several forms of learning. Although this might suggest that NMDA receptor-mediated processes are critical for synaptic plasticity, it is often difficult to rule out other mechanisms through which NMDA receptor antagonism might interfere with learning. For instance, the blockade of fear conditioning by microinfusion of the NMDA receptor antagonist AP5 into the basolateral amygdala (BLA) could occur if AP5 interfered with routine synaptic transmission, thus reducing the ability of stimuli to activate amygdala neurons. In second-order fear conditioning, the reinforcer is a fear-eliciting conditioned stimulus (CS), rather than an unconditioned stimulus (US). Expression of conditioned fear is amygdala-dependent, and thus provides a behavioural assessment of the ability of the reinforcer to activate amygdala neurons in the presence of AP5. We have now found that intra-amygdala AP5 actually enhanced expression of conditioned fear to the CS that provided the reinforcement signal for second-order conditioning. Nevertheless, acquisition of second-order fear conditioning was completely blocked. These findings strongly support the view that NMDA receptors are critically involved in synaptic plasticity.

f.) Normal conditioned inhibition and extinction of freezing and fear potentiated startle following electrolytic lesions of medial prefrontal cortex

The role of medial prefrontal cortex (mPFC) in the inhibition of conditioned fear was investigated, using both Pavlovian extinction and conditioned inhibition paradigms. In Experiment 1, lesions of ventral mPFC did not interfere with conditioned inhibition of the fear-potentiated startle response. In Experiment 2, lesions made after acquisition of fear conditioning did not retard extinction of fear to a visual conditioned stimulus (CS), and did not impair "reinstatement" of fear following unsignaled presentations of the unconditioned stimulus (US). In Experiment 3, lesions made prior to fear conditioning did not retard extinction of fear-potentiated startle or freezing to an auditory CS. In both Experiments 2 and 3, extinction of fear to contextual cues was also unaffected by the lesions. These results indicate that ventral mPFC is not essential for the inhibition of fear under a variety of circumstances.

g.) Role of the septum in the excitatory effect of corticotropin releasing hormone(CRH) on

the acoustic startle reflex.

Intracerebroventricular (i.c.v.) administration of CRH elicits a constellation of behavioral, autonomic, and endocrinological changes typically observed in stress. One of the behavioral changes following i.c.v. CRH is a profound increase of startle amplitude (CRH-enhanced startle). The present study examined the role of the septum in CRH-enhanced startle. The septum has direct and indirect connections to the amygdala and inhibits the amygdala. Electrophysiological data show that CRH in the septum is inhibitory. Therefore, it has been hypothesized that i.c.v. CRH inhibits the septum, which in turn disinhibits the amygdala, resulting in a constellation of changes via activation of amygdala efferent targets. In testing this hypothesis, it was found that electrolytic lesions of the medial septum, but not the lateral septum, blocked CRH-enhanced startle. However, fiber sparing chemical lesions of the medial septum did not block CRH-enhanced startle, suggesting that the blockade seen with the electrolytic lesions was caused by damage to fibers of passage. A major fiber bundle passing through the medial septum is the fornix, the primary efferent pathway for the hippocampus. Fimbria transection blocked CRH-enhanced startle almost completely, whereas the large electrolytic lesions of the dorsal hippocampus did not block CRH-enhanced startle. Taken together, these data suggest that perhaps the ventral hippocampus and its efferent target areas which communicate via the fimbria may be critically involved in CRH-enhanced startle.

h.) Role of the hippocampus, the bed nucleus of the stria terminalis and the amygdala in the excitatory effect of corticotropin releasing hormone(CRH) on the acoustic startle reflex.

Previously, we demonstrated that transection of the fimbria-fornix blocked the excitatory effect of CRH on startle (CRH-enhanced startle), suggesting that the hippocampus and its efferent target areas which communicate via the fimbria may be critically involved in CRH-enhanced startle. The bed nucleus of the stria terminalis (BNST) receives direct projections from the ventral hippocampus via the fimbria/fornix. Therefore, the role of the ventral hippocampus, the BNST, and the amygdala in CRH-enhanced startle was investigated. NMDA lesions of the BNST completely blocked CRH-enhanced startle whereas chemical lesions of the ventral hippocampus and the amygdala failed to block CRH-enhanced startle. However, the same amygdala lesioned animals showed a complete blockade of fear-potentiated startle, a conditioned fear response sensitive to manipulations of the amygdala. In contrast, BNST lesioned rats had normal fear-potentiated startle. This indicates a double dissociation between the BNST and the amygdala in two different paradigms which enhance startle amplitude. Micro-infusions of CRH into the BNST, but not into the ventral hippocampus, mimicked i.c.v. CRH effects. Furthermore, infusion of a CRH antagonist into the BNST blocked CRH-enhanced startle in a dose-dependent manner. Control studies showed that this blockade did not result from either leakage of the antagonist into the ventricular system or a local anesthetic effect caused by infusion of the antagonist into the BNST. The present studies strongly suggest that CRH in the cerebrospinal fluid can activate the BNST, which could lead to activation of brainstem and hypothalamic BNST target areas involved in anxiety and stress responses.

i.) Involvement of the dorsal periaqueductal gray in the loss of fear-potentiated startle

accompanying high footshock training

The amplitude of acoustic startle is markedly enhanced when elicited in the presence of cues signaling moderately intense footshock but, surprisingly, not when elicited in the presence of cues signaling higher intensity shocks. Previous results suggest that the loss of potentiated startle in rats trained with high footshock may involve activation of the dorsal periaqueductal gray (PAG). To evaluate this possibility, rats were trained with moderate (0.6 mA) footshocks and were subsequently tested following intra-PAG infusion of an excitatory nontoxic dose of kainic acid. Kainic acid significantly reduced fear-potentiated startle relative to vehicle controls. In a second experiment, the effects of dorsal PAG lesions on fear-potentiated startle to cues paired with 0.6 and 1.6 mA footshocks were evaluated. PAG lesions prevented the disruptive effects of high footshock training. Together, these results suggest that activation of the dorsal PAG mediates the loss of potentiated startle accompanying high-footshock training.

2. Publications:

a. Peer Reviewed

Lee Y, Walker D, Davis M: Lack of a temporal gradient of retrograde amnesia following NMDA-induced lesions of the basolateral amygdala assessed with the fear-potentiated paradigm. *Behavioral Neuroscience* 110:836-839, 1996

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McNish K, Davis M: The effects of olfactory bulbectomy on the acoustic startle reflex and its modulation by stress. *Behavioral Neuroscience* 111:80-91, 1997

Falls W A, Davis M: Inhibition of fear-potentiated startle can be detected after the offset of a feature trained in a serial feature negative discrimination. *Journal of Experimental Psychology: Animal Behavioral Processes* 23:3-14, 1997

Campeau S, Falls WA, Cullinan WE, Helmreich DL, Davis M, Watson SJ: Elicitation and reduction of fear: Behavioral and neuroendocrine indices and brain induction of the immediate-early gene *c-fos*. *Neuroscience* 78:1087-1104, 1997

Morgan C A, Grillon C, Southwick SM, Davis M, Charney DS: Exaggerated acoustic startle in gulf war veterans with post traumatic stress disorder. *American Journal of Psychiatry* 153:64-68, 1997

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- hormone on the acoustic startle reflex. *Journal of Neuroscience* 17: 6424-6433, 1997.
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b. Books and chapters

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Davis M, Walker DL, Lee Y: Roles of the amygdala and bed nucleus of the stria terminalis in fear and anxiety measured with the acoustic startle reflex: Possible relevance to PTSD. Annals of the New York Academy of Sciences, Psychobiology of Posttraumatic Stress Disorder, Vol. 821, The New York Academy of Sciences, New York, New York, 1997.

Davis, M. The neurophysiological basis of acoustic startle modulation: Research on fear motivation and sensory gating. In: Attention and Orienting: Sensory and Motivational Processes, (P.J. Lang, R.F. Simons & M. T. Balaban, Eds.), Lawrence Erlbaum, Associates: Hillsdale, N.J., 1997, pp. 69-96.

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